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Endothelins inhibit cyclic-AMP induced renin gene expression in cultured mouse juxtaglomerular cells

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Endothelins inhibit cyclic-AMP induced renin gene expression in cultured mouse juxtaglomerular cells. We have recently described that endothelins-1 to -3 equipotently inhibit cAMP stimulated renin secretion from cultured mouse juxtaglomerular cells by a process involving phospholipase C activation. This study examined the influence of endothelin-2 on renin gene expression in renal juxtaglomerular cells. To this end we semiquantitated renin mRNA levels by competitive RT-PCR in primary cultures of mouse renal juxtaglomerular cells after 20 hours of incubation. We found that endothelin-2 (0.1 to 100 nmol/liter) did not change basal renin gene expression. The adenylate cyclase activator forskolin (3 μmol/liter) increased renin mRNA levels to 400% of the controls and this stimulation was dose-dependently attenuated by ET-2 to 250% of the control value. The effect of ET-2 was mimicked by the ET_B-receptor agonist sarafotoxin S6c. The kinase inhibitor staurosporine (100 nmol/liter) increased renin secretion and renin mRNA levels. Combination of staurosporine with forskolin produced the same effects on renin secretion and renin mRNA levels as did staurosporine alone. In the presence of both forskolin and staurosporine ET-2 had no significant effect on renin secretion and renin gene expression. The phorbol ester PMA (30 nmol/liter), which was used to stimulate protein kinase C activity, attenuated cAMP stimulated renin secretion and renin mRNA levels. Lowering the extracellular concentration of calcium by the addition of 1 mmol/liter EGTA did not inhibit the effect of ET-2 on cAMP induced renin secretion and renin gene expression. These findings suggest that endothelins inhibit cAMP stimulated renin gene expression by an event that is mediated via ET_B receptors. This inhibitory effect may in part involve protein kinase C activation.

Endothelins (ETs) comprise a group of three related peptides (ET-1, -2, -3) that are known as potent vasoconstrictors [1]. Endothelins have also been found to potently influence renin secretion, the effect being dependent on the experimental model studied. *In vivo* both increases and decreases of plasma renin activity have been observed after the administration of endothelins [2, 3]. In cell cultures of human decidual cells ETs stimulate prorenin secretion [4], while they inhibit renin secretion from isolated kidneys [5, 6], kidney slices [7, 8], isolated rat glomeruli [9] and cell cultures of renal juxtaglomerular (JG) cells [8, 10], suggesting that the effect of ETs on renin secretion is tissue specific. For renal JG cells, evidence has been found that inhibition of renin secretion by ETs is rather specific for renin secretion stimulated by cyclic AMP [11] and that calcium is required for the inhibitory effect of ETs [8, 10, 11]. While an effect of ETs on renin secretion thus appears to be well established it is less known whether ETs also have influence on the expression of the renin gene. Since renal JG cells directly adjoin vascular endothelial cells [12], which are a major source of ETs, such a local control of renin gene expression by the endothelium via ETs would be conceivable. In cultures of decidual cells ETs clearly stimulate the expression of the renin gene, and further evidence suggests that this stimulation involves an activation of protein kinase C [13]. Whether ETs have influence on the renin gene in the kidney is less clear. A recent *in vivo* study indicates that administration of an ET antagonist does not change renal renin mRNA levels in normal rats and in rats with unilateral renal artery clips [14], suggesting that at least basal tissue levels of ETs are not important determinants of renin gene expression in the kidney.

We were therefore interested to find out whether ETs are capable of modulating renal renin gene expression. To this end we have determined the influence of ETs on renin mRNA levels in primary cultures of mouse renal JG cells. Our results suggest that ETs inhibit renin gene expression stimulated by cyclic AMP, but not basal renin gene expression, probably by an effect that involves protein kinase C activity.

Methods

Isolation and primary culture of mouse JG cells

Mouse juxtaglomerular cells were isolated as described previously [15]. For one cell preparation, four male C57Bl6 mice (4- to 6-weeks old) that had free access to normal food (Altromin, Lage, Germany) and water were killed by decapitation. The kidneys were removed, decapsulated, and minced with a scalpel blade. The minced tissue was incubated with gentle stirring in buffer 1 [130 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 10 mM glucose, 20 mM sucrose, 10 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 7.4], supplemented with 0.25% trypsin (Sigma, Deisenhofen, Germany) and 0.1% collagenase (Boehringer Mannheim, Germany) at 37°C for 70 minutes. After enzymatic dissociation, the tissue was sieved over a 22 μm screen. Single cells passing the screen were collected, washed, and resuspended in 4 ml of buffer 1 and then further separated using Percoll (Pharmacia, Uppsala, Sweden) density gradients. The cell suspension obtained was added to two tubes each containing 30 ml of 30% isosmotic Percoll in buffer 1. After 25 minutes of centrifugation at 4°C and 27.000 g, four cell layers with different specific renin activity were obtained. The cellular layer (density = 1.07 g/ml) with the highest specific renin activity was used for cell culture.

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These cells were washed in buffer 1 and resuspended in RPMI-1640 medium (Biochrom, Berlin, Germany) containing 0.66 U/ml insulin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2% fetal calf serum (FCS). The cultures were distributed in 100 μl aliquots into 96-well plates. The cultures were incubated at 37°C in humified atmosphere containing 5% CO_2 in air.

After 20 hours of primary culture, the culture medium was removed, and the cultures were washed once with 100 μl RPMI-1640 medium containing 2% FCS. Then 100 μl of fresh and prewarmed culture medium with the chemicals to be tested were added.

Experiments on renin secretion

Experiments on renin secretion were performed for 20 hours of incubation. At the end of experiments supernatants were collected and centrifuged at 1,000 g at room temperature to remove cellular debris. The supernatants were then stored at −20°C until assayed for renin activity. Cells were lysed by adding to each culture well 100 μl of phosphate-buffered saline (PBS) containing 0.1% of Triton X-100 and shaking for 45 minutes at room temperature. The lysed cells were stored at −20°C until further processing.

Renin secretion rates were estimated from the appearance rate of renin in the culture medium. To minimize differences among different cell culture preparations, renin secretion rates were calculated as fractional release of total renin [renin activity released/(renin activity released + renin activity remaining in the cells)].

Renin activity was determined by its ability to generate angiotensin (Ang) I from the plasma of bilaterally nephrectomized rats [16]. Ang I was measured by radioimmunoassay (Sorin Biomedica, Düsseldorf, Germany).

Measurement of renin mRNA by quantitative RT-PCR

RT-PCR experiments were performed as described [17].

Construction of an internal standard

Choice of primers. To avoid coamplification of genomic DNA coding for renin, two oligonucleotide primers, one spanning the exon 6/exon 7 border and the other located on exon 8 of the renin gene, were chosen, thus amplifying a 194-bp sequence. Sense primer (5′-ATG AAG GGG GTG TCT GTG GGG TC-3′) and antisense primer (5′-ATG TCG GGG AGG GTG GGC ACC TG-3′) were synthesized with a PCR-mate DNA synthesizer (Applied Biosystems Inc, Foster City, CA, USA). Both primers were checked for the absence of fortuitous homology to other known sequences in the GenBank data base.

Internal standard preparation. An internal standard was synthesized by *in vitro* transcription from plasmid pISMR. This plasmid was constructed from clone pRn 1 to 2: complete renin cDNA 1427 nucleotides in length was isolated using *Pst*I restriction sites and subcloned into the *Pst*I restriction site of Bluescript BS-SK (Stratagene Inc, La Jolla, CA, USA). Subsequently, a 60-bp linker fragment corresponding to *Hin*dIII/*Sac*I polylinker derived from BS-SK was inserted into blunt-ended *Bcl*I site renin cDNA (942 nucleotides). The *Bcl*I restriction site is in the amplified renin fragment. Renin cRNA was prepared as a sense strand from the *Sac*I- digested pISMR template using T7 RNA ploymerase according to the *in vitro* transcription protocol (Stratagene). After the reaction, the template was completely digested with RNase-free DNase I. Renin cDNA could not be amplified in the absence of reverse transcriptase, thus confirming that the internal standard only consisted of renin RNA.

RT reaction on cultured cells: Cell lysate preparation. At the end of the incubation experiments, primary cultured cells were placed on ice, the culture medium was removed, and the cultures were washed once with ice-cold buffer 1. Then, cells were lysed by the addition of 40 μl of an ice-cold buffer containing 2% Nonidet (NP 40), 10 mM Tris-HCl (pH 8), 10 mM NaCl, 3 mM $MgCl_2$, and 0.7% (vol/vol) mercaptoethanol to each culture well and shaking for 15 minutes at 4°C. The cellular lysates of two culture wells were pooled for the determination of renin mRNA. Cell nuclei were removed by centrifugation at 12,000 g and 4°C for three minutes. Three microliters of the cytoplasmatic fraction was immediately used for reverse transcription.

RT reaction. Ten microliters of an ice-cold solution containing 10 pM of the antisense primer, 1 μg of yeast tRNA, and 5 pg of the internal standard was added to 3 μl of the cytosolic fraction. Samples were heated for five minutes at 65°C and then chilled on ice. Then, 10 μl of solution A were added, and the samples were incubated for one hour at 37°C. Solution A consisted of 22 μl of a 25 mM solution of deoxyribonucleotides, 45 μl of 5x RT buffer (supplied with the RT kit, GIBCO-BRL), 6 μl of bovine serum albumin (20 mg/ml; Boehringer), 6 μl of the RNase inhibitor rRNasin (40,000 U/ml; Promega, Serva, Heidelberg, Germany), 1.1 μl murine-Moloney leukemia virus RT (200 U/μl, GIBCO-BRL), and 20 μl of 0.1 mM dithiothreitol. After incubation the reaction was stopped by heating the samples for two minutes at 95°C.

PCR. To 3 μl of the cDNA solution, 2.5 μl of 10xPCR buffer supplied with *Taq* polymerase (Boehringer), 1 μl (10 pM) of each primer, 4.5 μl of a 25 mM $MgCl_2$ solution, 14 μl H_2O, and 10 μCi of [^{3}H]dCTP were added. Samples were overlayered with mineral oil, denaturated at 94°C for five minutes, and cooled to 65°C for five minutes. After addition of 1 μl of 25 mM deoxyribonucleotide solution and 1.25 U *Taq* polymerase, 35 PCR cycles consisting of denaturation at 94°C (1 min), annealing at 60°C (1 min), and extension at 72°C (30 seconds) were performed. PCR was completed by a final extension step of 10 minutes at 72°C.

After PCR, the amplification products originating from renin mRNA or from the internal standard were separated by polyacrylamide gel electrophoresis. N,N′-methylene-bis-acrylamide was replaced by dihydroxy-ethylene-bis-acrylamide. The bands were excised, solubilized in 0.025 M periodic acid at 65°C, and counted in a beta counter. The radioactivity incorporated by renin mRNA was routinely expressed as a percentage of the radioactivity incorporated by the 5 pg of internal standard. Absolute values for renin mRNA were extrapolated from the theoretical correction factor of 1.3 accounted for the minor size of renin mRNA (194 bp) compared with the internal standard (254 bp).

Chemicals

Culture media and trypsin-EDTA were purchased from Biochrom. Endothelin-2, forskolin, staurosporine, phorbol ester phorbol-12-myristate-13-acetate (PMA), and EGTA were obtained from Sigma. Sarafotoxin S6c were purchased from Alexis (Läufelfingen, Switzerland).

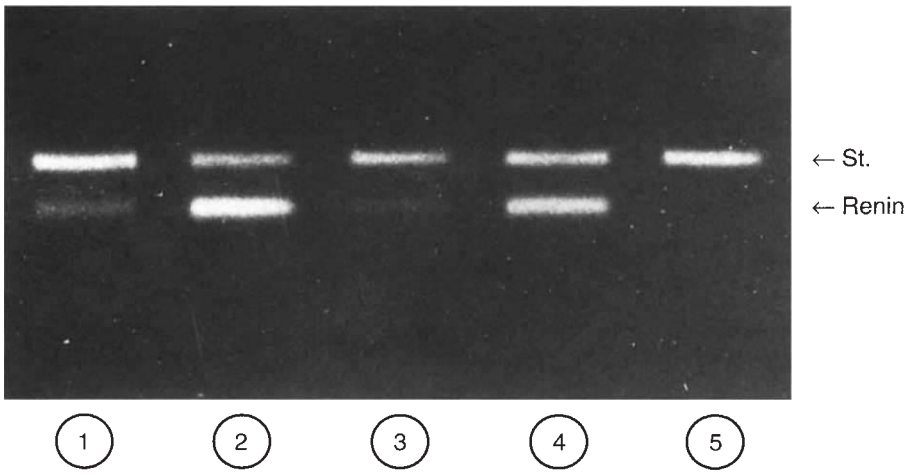

Fig. 1. *Agarose gel separating PCR products utilizing internal renin RNA standard (St) and RNA form cultured mouse renal juxtaglomerular cells.* The bands were visualized by ethidium bromide staining: (1) control, (2) forskolin 3 μM, (3) endothelin-2 10 nM, (4) forskolin + endothelin-2, (5) internal RNA standard alone.

Statistics

If not otherwise indicated, a total number of three different kidney preparations was taken for each experimental protocol. Data on renin secretion are given as means ± SEM, and each experiment was performed in quadruplicate cultures. Levels of significance were calculated using the Student's *t*-test. $P < 0.05$ was considered significant.

Results

This study aimed to assess the effect of endothelins on renin gene expression in cultured mouse JG cells. Since we previously have found that the effect of endothelins in these cells is predominantly mediated by endothelin ET_B-receptors and all three endothelin peptides act equipotently [11], we selected ET-2 to examine the effects of endothelins in this study. Moreover, we found previously that ETs preferentially inhibit cAMP stimulated renin secretion [11]. This was the reason to also study the effect of ET-2 on renin gene expression in the presence of the adenylate cyclase activator forskolin (3 μmol/liter).

Figure 1 shows a representative agarose gel separating PCR products of the internal renin mRNA standard from that of renin mRNA isolated from primary cultures of mouse renal JG cells under various experimental conditions. It is already obvious from the gel that the renin mRNA band of cultures treated with ET-2 (10 nmol/liter) is not strikingly different from the control cultures. Treatment of cultures with the adenylate cyclase activator forskolin (3 μmol/liter) produced a stronger band than that of control cultures and ET-2 (10 nmol/liter) decreases the intensity of the band obtained with forskolin.

A more quantitative analysis of the effects of forskolin and of ET-2 on renin mRNA levels expressed relative to the control cultures is shown in Figure 2. It can be seen that ET-2 in a concentration range between 0.1 to 10 nmol/liter dose dependently attenuated forskolin-induced renin secretion (Fig. 2A) and renin mRNA levels (Fig. 2B). Notably, ET-2 did not fully abolish the effect of forskolin on renin secretion and renin mRNA levels.

The half maximal effect of ET-2 occurred between 1 and 10 nmol/liter.

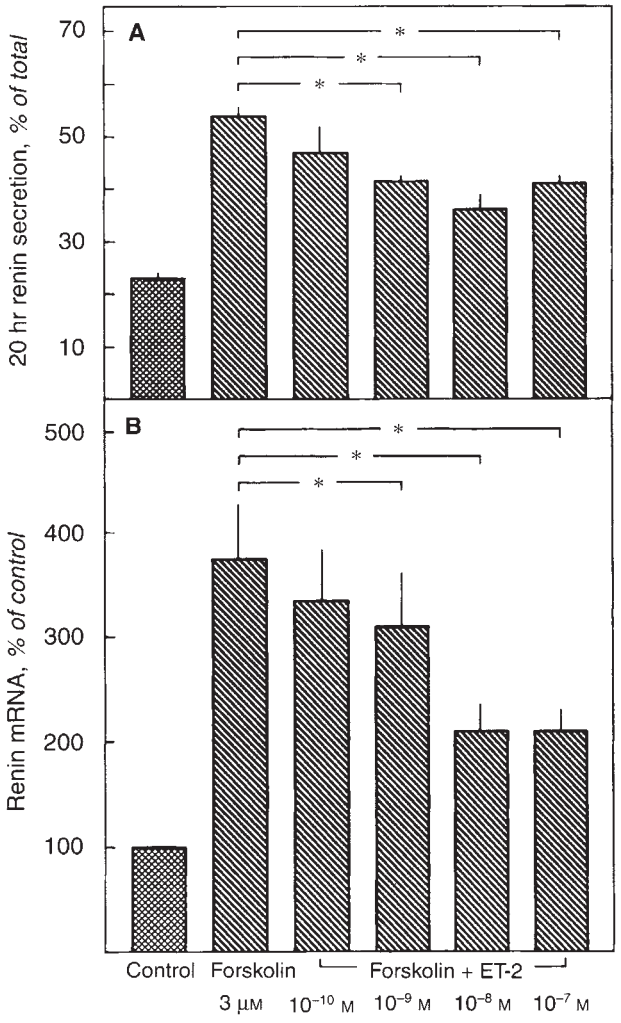

Fig. 2. *Dose-dependent effects of ET-2 on forskolin-stimulated renin secretion* (**A**) *and on renin mRNA levels* (**B**) *of cultured mouse JG cells during 20 hours of incubation.* Data are means ± SE of 4 experiments. Each experiment on renin secretion represents mean of quadruplicate cultures. *$P < 0.05$.

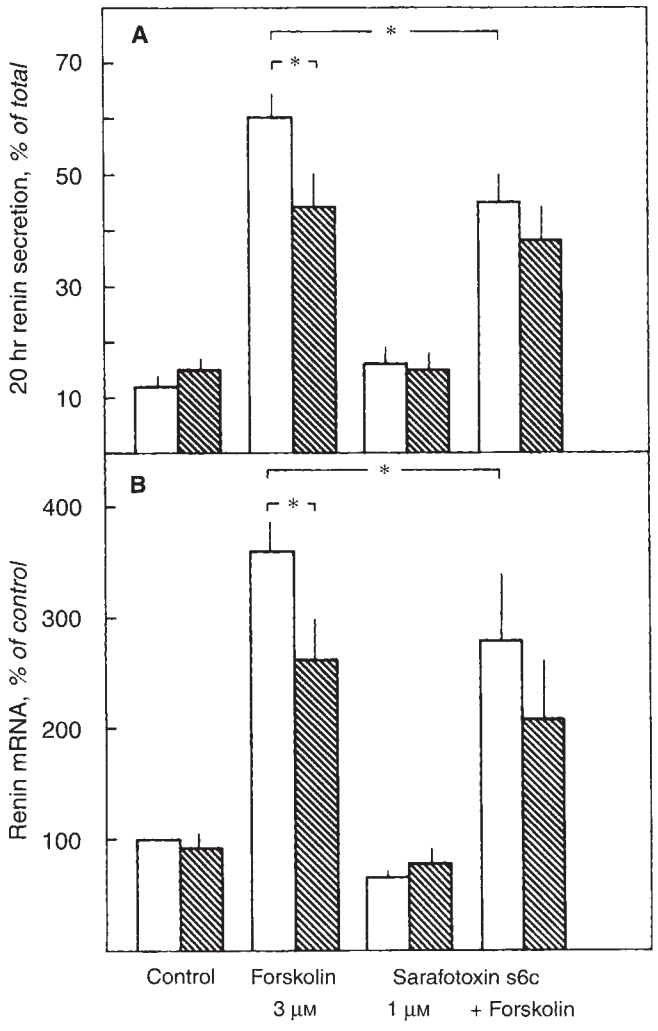

Fig. 3. *Effects of ET-2 (10 nM) and sarafotoxin S6c (1 μM) on basal and cAMP-stimulated renin release* (**A**) *and on renin mRNA levels* (**B**) *of isolated juxtaglomerular cells during 20 hours of primary culture.* Data are means ± SE of 3 experiments. Each experiment on renin release represents mean of quadruplicate cultures. Symbols are: (▨) with ET-2 10 nM; (□) without ET-2 10 nM. $^*P < 0.05$.

Since we found previously that the effects of endothelins on renin secretion in renal JG cells is predominantly mediated by ET_B-receptors [11] we compared the effects of ET-2 and of sarafatoxin (S6c), which is considered as a preferential ligand for ET_B-receptors [18]. As shown in Figure 3 sarafotoxin S6c had almost the same potency to attenuate cAMP stimulated renin gene expression and renin secretion as had ET-2.

Endothelins are known as potent activators of phospholipase C in their target cells leading to activation of protein kinase C and intracellular calcium mobilization. This happens also in renal JG cells [11]. We therefore tested whether these second messenger pathways were involved in the inhibitory effect of ET-2 on cAMP-induced renin gene expression and renin secretion.

To examine a possible involvement of calcium in the inhibitory effect of ET on cAMP stimulated renin gene expression we lowered the extracellular concentration of calcium to about 80 and 250 nmol/liter by the addition of 2 or 1 mmol/liter EGTA to the culture medium.

As previously noted, EGTA at 2 mmol/liter abolished the effect of ET-2 on forskolin stimulated renin secretion [11], but at the same time led to a rapid break down of general transcription including renin gene transcription [19], and was therefore not considered further. At 1 mmol/liter EGTA did not abolish the inhibitory effect of ET-2 on cAMP-induced renin secretion but significantly attenuated forskolin-stimulated renin mRNA levels in the cultured cells (Fig. 4). The inhibitory effect of ET-2 on forskolin-stimulated renin mRNA, however, was still apparent under this condition (Fig. 4B).

To obtain information about a possible involvement of protein kinase C (PKC) activity, the effect of the kinase inhibitor staurosporine (100 nmol/liter), considered to preferentially inhibit PKC activity, was examined [20]. As shown in Figure 5 staurosporine alone had a significant stimulatory effect on renin mRNA levels and on renin secretion during 20 hours of incubation. In the presence of staurosporine forskolin did not further increase renin mRNA levels nor renin secretion (Fig. 5). Similar effects were also seen with a lower concentration (30 nmol/liter) of staurosporine and with the kinase inhibitor H7 (not shown). Both in the presence of either staurosporine alone or in the combined presence of staurosporine and forskolin, ET-2 failed to lower renin mRNA levels and renin secretion (Fig. 5). We also used sphingosine as a more specific inhibitor of protein kinase C activity. At a concentration 20 μmol/liter totally blocked renin gene expression and this effect went in parallel with an inhibition of general RNA synthesis. At 2 μmol/liter sphingosine had neither influence on renin secretion nor renin gene expression. Sphingosine was therefore not considered further.

To stimulate PKC activity directly the phorbol ester phorbol-12-myristate-13-acetate (PMA) was used. As shown in Figure 6 PMA at a concentration of 30 nmol/liter did not significantly change basal renin mRNA levels nor renin secretion, but significantly attenuated renin mRNA and renin secretion stimulated by forskolin. The combination of PMA (100 nmol/liter) with ET-2 (10 nmol/liter) did not further increase the inhibitory effect of either ET or PMA alone (Fig. 6).

A last set of experiments was done to examine whether the effects of endothelin on renin secretion and renin gene expression were due to interference with cAMP formation rather than with the intracellular action of cyclic AMP. To this end the effect of the membrane permeable cAMP analogue 8-bromo-cAMP was examined. 8-br-cAMP (3 mmol/liter) increased both renin secretion (Fig. 7A) and renin mRNA levels (Fig. 7B), albeit to a substantially smaller extent than did forskolin. ET-2 (10 nmol/liter) significantly attenuated both renin secretion and renin mRNA levels stimulated by 8-br-cAMP (Fig. 7).

Discussion

It is well established that endothelins act as inhibitors of renin secretion from the kidneys [5–9, 21]. Our previous findings suggest that this effect is mediated by ET_B rather than by ET_A receptors on renal juxtaglomerular cells [11]. In this study we also found that endothelins inhibit cAMP-stimulated renin secretion but are less effective on basal secretion. The primary goal of this study was to find out whether endothelins also influence the expression of the renin gene in renal juxtaglomerular cells, and in fact our

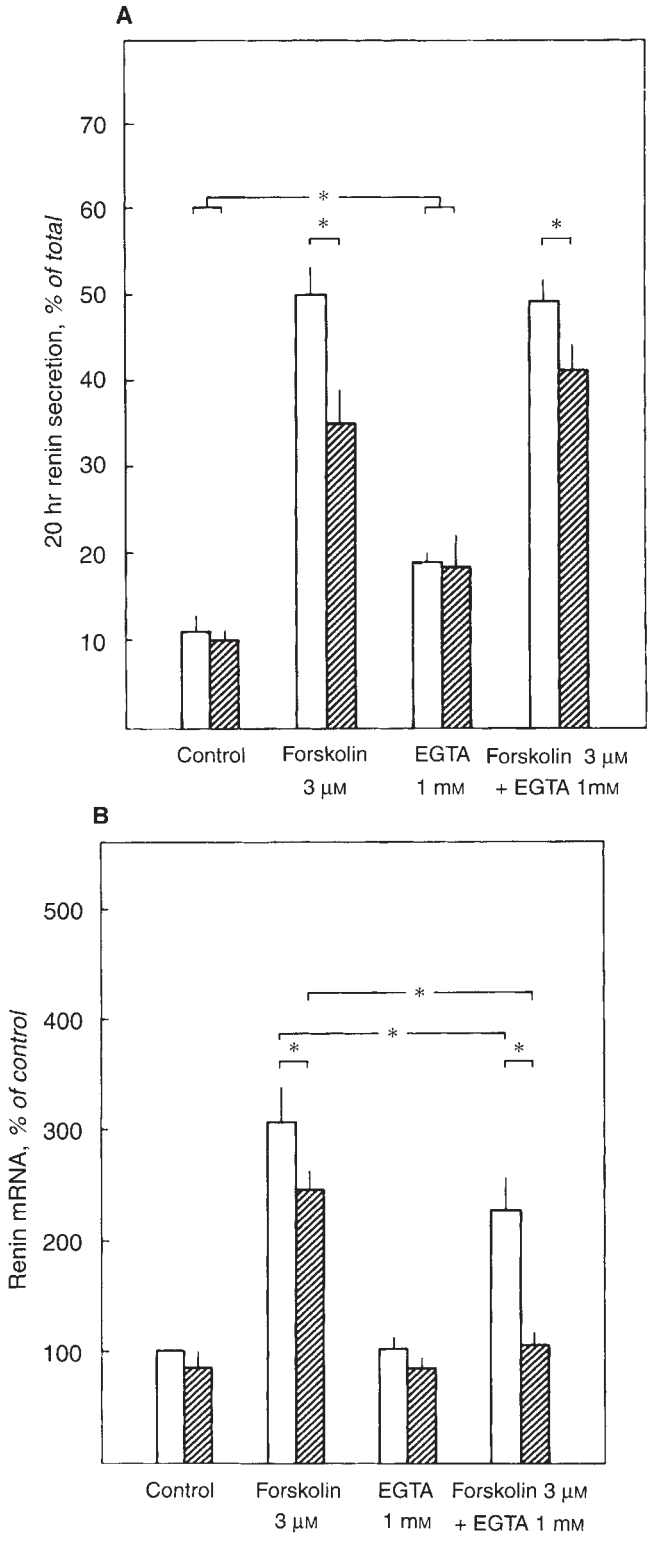

Fig. 4. *Effect of lowering extracellular Ca^{2+} with 1 mM EGTA on basal and forskolin-stimulated renin secretion* (**A**) *and on renin mRNA* (**B**) *levels in the presence of endothelin-2 in juxtaglomerular cells during 20 hours of primary culture.* Data are means ± SE of 4 experiments. Each experiment on renin secretion represents mean of quadruplicate cultures. Symbols are: (▨) with ET-2 10 nM; (□) without ET-2 10 nM. * $P < 0.05$ versus control.

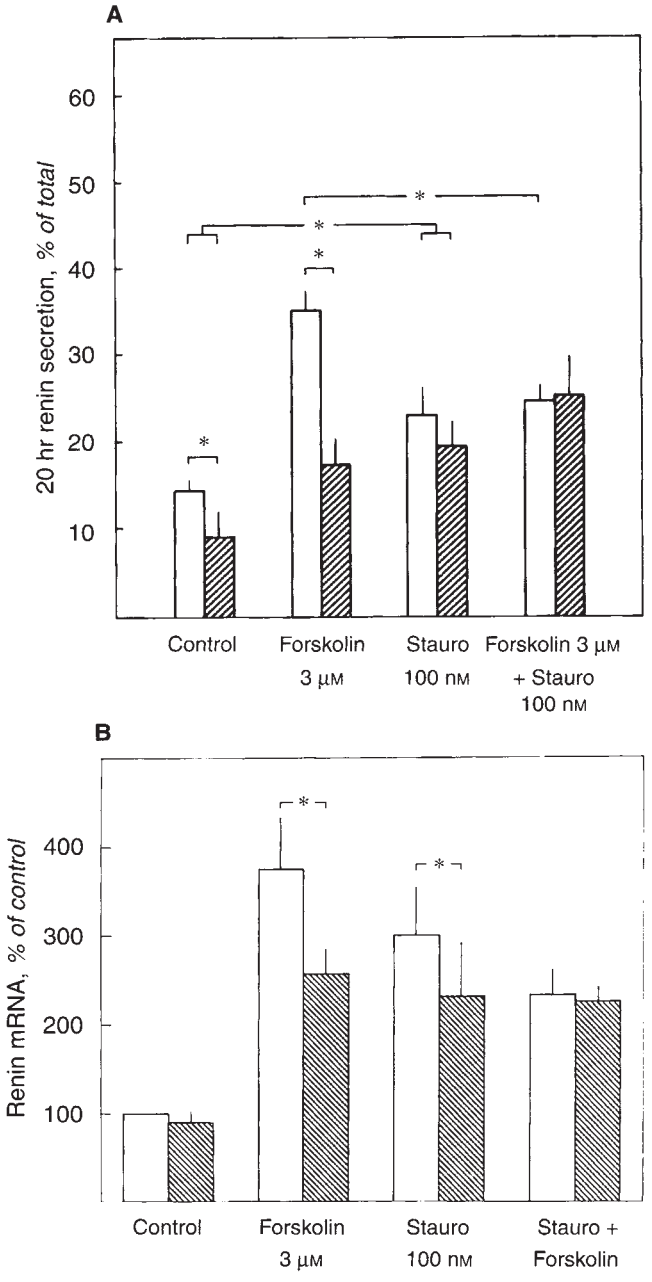

Fig. 5. *Effect of staurosporine (100 nM) on renin secretion* (**A**) *and on renin mRNA levels* (**B**) *in cultured JG cells during 20 hours of incubation.* Data are means ± SE of 5 experiments. Each experiment on renin secretion represents mean of quadruplicate cultures. Symbols in A are: (▨) with ET-2 10 nM; (□) without ET-2 10 nM. Symbols in B are: (▧) with ET-2 100 nM; (□) without ET-2 100 nM. * $P < 0.05$ versus control.

finding suggest that they do so. Similar to their effects on renin secretion ET-2 and the ET_B-receptor agonist, sarafotoxin S6c inhibited cAMP-stimulated renin gene expression but had no inhibitory effect on basal renin mRNA levels. As with secretion ET-2 only partially inhibited cAMP-stimulated renin gene expression and as already discussed previously [11], the reasons for this partial inhibition of cAMP stimulation by endothelin are not yet

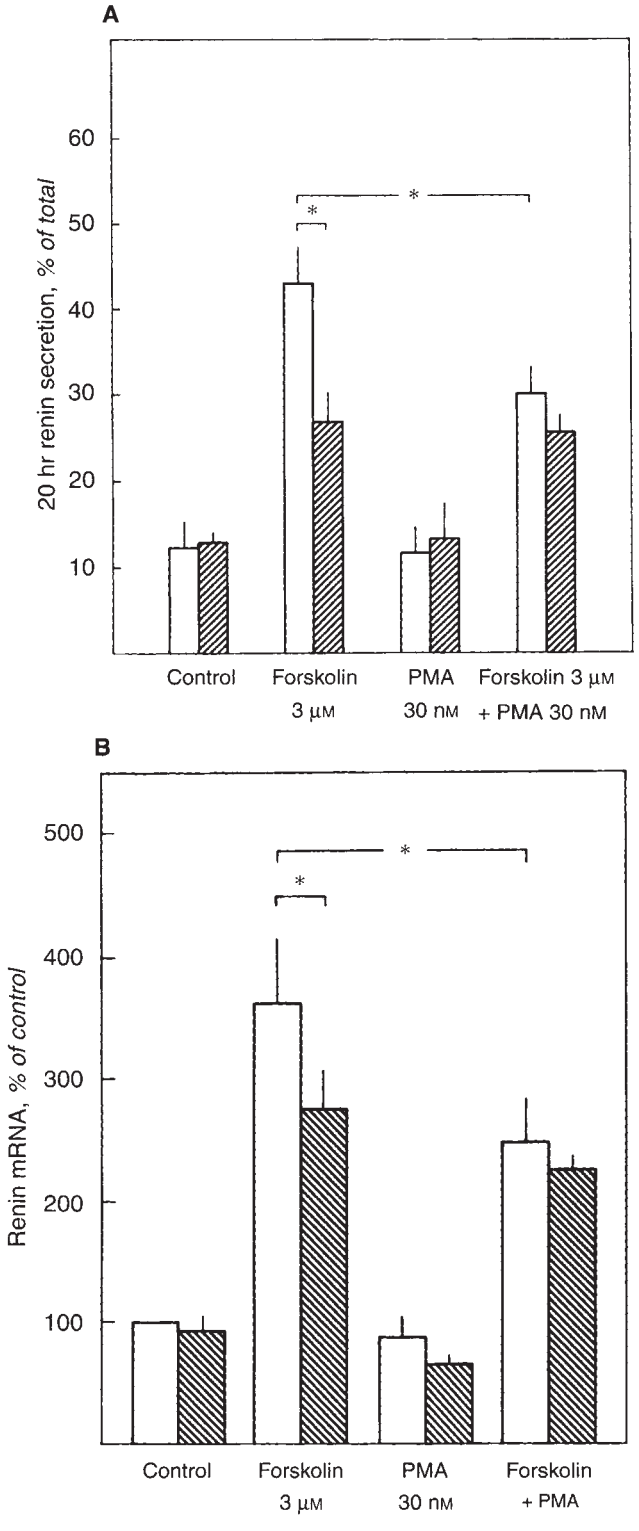

Fig. 6. *Combined effects of endothelin-2 (10 nM) and PMA (30 nM) on forskolin-stimulated renin secretion* (**A**) *and renin mRNA levels* (**B**) *in isolated cultured juxtaglomerular cells.* Data are means ± SE of 3 experiments. Each experiment on renin secretion represents mean of quadruplicate cultures. Symbols in A are: (▨) with ET-2 10 nM; (□) without ET-2 10 nM. Symbols in B are: (▧) with ET-2 10 nM; (□) without ET-2 10 nM. *$P < 0.05$ versus control.

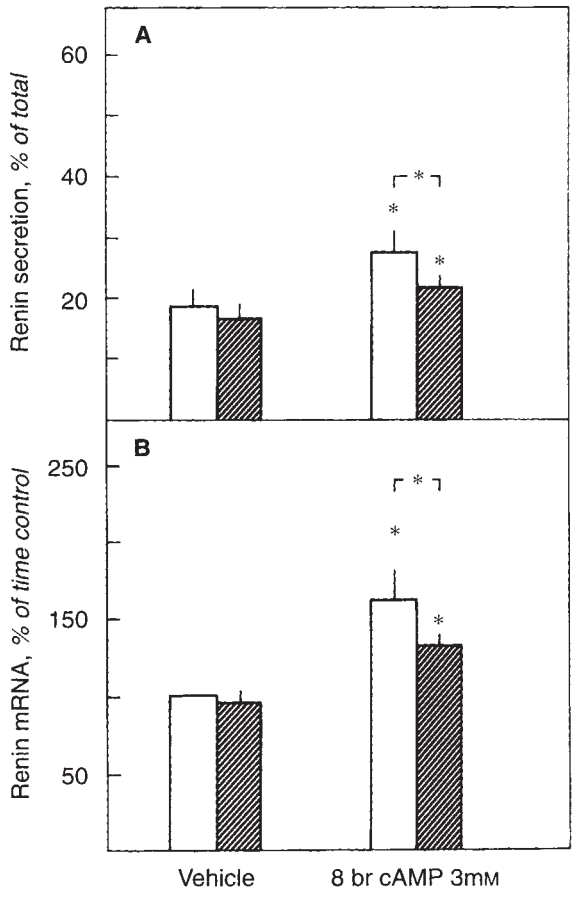

Fig. 7. *Effects of endothelin-2 (10 nM) on 8-br-cAMP stimulated renin secretion* (**A**) *and renin mRNA levels* (**B**) *in isolated cultured juxtaglomerular cells.* Data are means ± SE of five experiments. Each experiment on renin secretion represents mean of quadruplicate cultures. Symbols are: (▨) ET-2 10 nM; (□) control. *$P < 0.05$ versus respective controls.

clear, but appear not to be due to modulations of intracellular cAMP levels. This inference is supported by the findings that ET-2 also attenuated renin secretion and renin gene expression stimulated by the cAMP analogue 8-br-cAMP (Fig. 7).

To narrow down the possible pathways along which ET-2 could exert its inhibitory effect on cAMP-stimulated renin secretion, we focused on the possibile involvement of protein kinase C (PKC) activity and of calcium. Both are the classic messengers resulting from stimulation of phospholipase C activity, which is known to be activated by both ET-receptor types [22–24] also in JG cells [11].

In accordance with findings presented by others [8, 9], we have previously observed that the inhibitory effect of ETs on renin secretion is blunted if the extracellular concentration of calcium is lowered by the addition of 2 mmol/liter EGTA, suggesting that calcium is required for the inhibitory effect of ETs on cAMP-induced renin secretion [11].

This study confirmed our previous notion [19] that chelation of extracellular calcium by 2 mmol/liter EGTA has a detrimental effect on general transcriptional activity, and together with our previous observation that calmodulin antagonists also inhibit general protein synthesis [25] in cultured JG cells these findings

suggest that calcium/calmodulin mediated reactions are required for normal gene transcription in cultured JG cells. Lowering extracellular calcium to about 250 nmol/liter by the addition of 1 mmol/liter EGTA already attenuated the stimulation of renin gene expression by cAMP but did not prevent it. This concentration also significantly stimulated basal renin secretion but did not prevent the inhibitory effect of ET-2 on cAMP-induced renin gene expression nor on cAMP-stimulated renin secretion.

In view of the crucial effect of calcium for general transcription, it is very difficult to estimate the involvement of calcium in renin gene regulation from calcium depletion experiments. We can therefore not rule out that calcium is involved in the inhibitory effect of ETs on cAMP stimulated renin gene expression, a possibility that is conceivable from the findings that ETs lead to long lasting elevations of intracellular Ca^{2+} in JG cells [11] and that longer lasting elevations of cytosolic calcium attenuate cAMP-stimulated renin mRNA levels in cultured JG cells [19].

To examine the possible involvement of protein kinase C activity in the effects of ET-2 on JG cells we used staurosporine, which is a kinase inhibitor with a preferential action on protein kinase C [20]. Our findings show that staurosporine itself substantially increased renin secretion and renin mRNA levels. Given that this effect was due to kinase inhibition, this observation suggests that basal renin secretion and renin gene expression in cultured JG cells are under the inhibitory control of a kinase activity, probably PKC activity. That staurosporine also inhibited kinases different from PKC may explain the lack of forskolin to further stimulate renin secretion and renin gene expression, suggesting that probably also protein kinase A was affected by staurosporine in our experiments. Nonetheless, our findings show that that the inhibitory effect of ET-2 on renin mRNA was significantly diminished in the presence of staurosporine, suggesting that ET-2 requires intact kinase activity, probably PKC activity, to exert its full effect on renin gene expression stimulated by forskolin.

That activation of PKC could inhibit renin secretion and renin gene expression in juxtaglomerular cells is suggested by the effect of the active phorbol ester PMA, which attenuated forskolin stimulated renin secretion and renin gene expression. This effect of PMA is in accordance with our previous observation that PMA attenuates forskolin stimulated (pro)renin synthesis in cultured JG cells [15].

Taken together, our findings suggest that endothelins inhibit cAMP-stimulated renin gene expression via ET_B receptors. This inhibition could involve PKC activity but also other pathways such as an elevation of the cytosolic calcium concentration, which may act alone or in concert with protein kinase C activity. Moreover, our findings suggest that either PKC or another protein kinase exerts a potent inhibitory effect on renin secretion and renin gene expression in the basal state.

The role of endothelins and of PKC on the function of renal JG cells is thus apparently opposite to its role in decidual cells, which also produce and secrete prorenin. Endothelins are potent stimulators of prorenin secretion and renin gene expression in cultured decidual cells [4, 13]. Moreover, it has been found that PKC activity is stimulatory for renin gene expression in these cells and cAMP and PKC cause potentiating effects on renin mRNA levels. The particular role of PKC on renin gene expression thus appears to be tissue and stimulus dependent, indicating that cell specific pathways for the control of the renin gene exist.

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